REMARKS/ARGUMENTS

Reconsideration of this application and entry of the foregoing amendments are respectfully requested.

The claims have been revised to define the invention with additional clarity. The claims as presented are fully supported by an enabling disclosure.

Claim 4 stands rejected under 35 U. S. C. 112, first paragraph, as allegedly being non-enabled. Withdrawal of the rejection is submitted to be in order in view of the above-noted amendment of claim 4 and further in view of the comments that follow.

From a review of the Examiner's comments, it appears that the rejection is based on the recitation of "... which is therapeutic". That language does not appear in claim 4 as now presented.

The Examiner states that the term "therapeutic encompasses all diseases or conditions that afflict humans or animals". The Examiner also states that the fact "that one can successfully introduce kid/kis into cells does not in any way imply that a particular disease can be treated." Further, the Examiner states that "there is no reason to believe that selectively killing eukaryotic cells would be therapeutic for a disease caused by bacterial growth". Although the Examiner acknowledges that zebrafish might be a model for some human diseases, the Examiner contends that they have not been shown to be good models of diseases such as AIDS, Alzheimer's or gonorrhoea.

Claim 4 as amended requires that the target eukaryotic cells be in the human or animal body. Thus, it is clear that claim 4 is not directed to the treatment of <u>all</u> diseases or conditions which affect humans or animals but rather to the selective cell cycle inhibition and/or cell death

in the body. As explained in the application as filed, this may be beneficial in various contexts, e.g., in the context of hyperproliferative disorders.

The Examiner also states that Applicants "have not shown any way that particular cells ... in a developed organism could be targeted; nor ... what cells need to be targeted to treat any particular disease." The Examiner states that "the only *in vivo* methods shown by applicant have involved manipulation of one and two-celled embryos", and that "[i]t has not been shown that creating a transgenic human by manipulating one or two-celled embryos is effective in any therapeutic capacity."

Applicants respectfully submit that means for delivering proteins and/or nucleic acids to cells within the human and animal body are well known in the art. Moreover, methods of providing proteins preferentially within certain cells and tissues are well known. Examples include: localized delivery to the tissues (e.g., by injection); targeting to tissue-specific cell surface molecules; and expression under the control of tissue-specific promoters. Accordingly, the skilled person would be well aware of methods for targeting cells in a developed organism.

The Examiner further states that the art teaches "that while the use of toxins to control eukaryotic cells growth (especially cancer) is promising, there are hurdles to be overcome." Vassaux *et al* (Breast Cancer Res., 2:22-27, 20000) allegedly states that clinical use of genetic toxins would require efficient and reliable targeting of cancer cells, and this cannot be achieved by current tools. Fitzgerald *et al* allegedly states that two problems must be overcome to use toxins: firstly, administration of toxins to humans has lead to unanticipated toxicity in normal tissues, and secondly, the human immune response limits the effect of the toxins.

However, with regard to the effects of the human immune response, Fitzgerald *et al* only states that this limits the <u>duration</u> over which the toxins can be administered:

"Currently, the administration of chimeric toxins is restricted to a two week period before neutralising antibodies develop."

Hence, Fitzgerald *et al* does not teach that the skilled person would be prevented from obtaining the effect of controlling eukaryotic cell growth with toxins. Rather, at most this document teaches that the duration of a therapeutic treatment may be limited. Applicants respectfully submit this is irrelevant to the enablement of claim 4.

With regard to the need to target cancer cells and minimize possible toxicity in normal tissues, Applicants point out that the present invention is specifically concerned with addressing this problem in the use of toxins.

General methods of targeting toxins to particular cells/tissues are well known in the art. For example, and as noted above, suitable methods include: regulated expression under the control of cell- or tissue-specific promoter; using chimeric toxins targeted to cell surface molecules; or localized delivery e.g., by injection. However, the targeting may be "leaky" such that normal cells are also affected by the presence of low concentrations of the toxin.

According to the present invention, non-target cells are protected by the presence of an antitoxin. For instance, in the paper by Slanchev *et al* (2005) submitted with the previous response, systemic expression of the antitoxin negated the effects of "leaky" toxin expression in non-target tissues and thus protected the non-target tissues from damage.

Thus, the methods of the present invention provide selectivity to target cells, allowing proliferation to be inhibited in target cells while reducing damage to non-target cells.

The Examiner has acknowledged that Vassaux *et al* and Fitzgerald *et al* do not follow the method of the instant application. Thus, Applicants submit that it is improper to use difficulties expressed in these documents as basis for rejecting the present claims as non-enabled.

The Examiner is urged to reconsider the rejection in view of the above. It is earnestly believed that, having done so, the Examiner will find withdrawal of the rejection to be in order.

Claims 1-4, 10 and 12-16 stand rejected under 35 USC 112, first paragraph, because the specification, while being enabled for methods in a target eukaryotic cell *in vitro*, allegedly does not reasonably provide enablement for the methods as claimed. Withdrwal of the rejection is submitted to be in order for the reasons that follow.

In assessing the Wands factors, the Examiner has stated that, according to the art, administration of toxins to humans has lead to unanticipated toxicity in normal tissue, and secondly, the human immune response limits the effects of the toxin. Applicants comments responsive to these points are provided above. The teachings of Vassaux *et al* and Fitzgerald *et al* have no relevance to the enablement of the present claims. It is improper to base a rejection on these documents since they do not follow the method of the present invention.

Moreover, with regard to the Examiner's statement that the human immune response limits the effects of the toxin, it is respectfully noted that this cannot apply when the toxin and antitoxin are produced in the cell by expression from a nucleic acid. (In this embodiment, the toxin and antitoxin are not exposed to the immune system.)

The Examiner further states that the specification does not provide direct evidence for an *in vivo* benefit, or a reasonable basis for correlating the *in vitro* data as exemplified in the instant specification with *in vivo* benefit". The Examiner contends that "while those of skill in the art recognize that *in vitro* assays or cell-culture based assays are somewhat useful to observe basis physiological and cellular phenomenon such as screening the effects of potential drugs, clinical correlations are generally lacking." The Examiner also contends that the "increased complexity of the *in vivo* environment as compared to the very narrowly defined and controlled conditions of

an *in vitro* assay does not permit a single extrapolation of *in vitro* assays to *in vivo* efficacy with any reasonable degree of predictability." The Examiner states that "it is well known in the art that cultured cells, over a period of time, lose phenotypic characteristics associated with their normal counterpart cell type due to dissociation from a three-cell geometry and propagation on a two-dimensional substrate. The Examiner cites documents by Freshney and Dermer as allegedly describing the differences between cultured cells and cells *in vivo*.

Applicants maintain that the methods carried out on *Xenopus laevis* embryos described in the present application (which follow the embryos up to mid blastula transition) and the methods carried out in zebrafish as described in the paper by Slanchev *et al* (2005) are *in vivo* methods. Although the constructs are injected at the one-cell stage embryos, they are effective in the developing embryo, i.e., in a living, multicellular organism. Therefore, the Examiner's assertions to the contrary, the specification does contain direct evidence of effectiveness *in vivo*. Certainly, the methods carried out on *Xenopus laevis* embryos do not related to cells in culture and, thus, a rejection cannot be fairly based on differences between cell culture and cells *in vivo*.

In any case, Applicants respectfully submit that the test to be applied is whether there is a reasonable correlation between the utility disclosed in the application and the claimed activity (MPEP 2164.02). A rigorous or invariable exact correlation is not required. Furthermore, the Examiner is reminded that it is necessary to take due account of the nature of the invention, as set out in the Wands factors.

The present invention relates to a toxin/antitoxin system. The specification shows that the toxin prevents cell proliferation in eukaryotic cells. The specification further shows that the antitoxin retains the ability to neutralize the toxin in eukaryotic cells, and so can protect viability of eukaryotic cells in the presence of the toxin. The Examiner has provided no specific reason to

suppose that these effects are dependent on details of the cell environment, or on its cell-cell interactions.

The specification shows that the toxin/antitoxin system is effective in yeast, human and *Xenopus laevis* (frog) cells (*in vivo*). The paper by Slanchev *et al* (2005) shows that the toxin/antitoxin system is also effective in zebrafish (*in vivo*). Thus, the toxin/antitoxin system is effective in diverse eukaryotic cell types, and in a living multicellular organism. In view of this, there is no reason to suppose that the effect is sensitive to the specific nature or environment of the cells. Thus, the correlation between the data in the application and the claimed *in vivo* method is a reasonable one (MPEP 2164.03).

Finally, the Examiner contends that neither the Applicants nor the art have/has shown a predictable method of delivering the kid/kis genes to multiple cells or to specific cells in a developed organism so that the target cells can be controlled. The Examiner states that "it has not been shown how to target cells in the developed organism". The Examiner also states that "[t]he only *in vivo* methods shown by applicant have involved manipulation of one or two-celled embryos." The Examiner contends that this can not be considered an *in vivo* method, since while these embryos do grow into organisms, it has not been shown how to target cells in the developed organism. Further, it has allegedly not been shown that creating a transgenic human by manipulating one or two-celled embryos would lead to predictable results. The Examiner also states that the claims encompass delivery of kid/kis proteins by themselves, not only by delivery of the kid/kis genes, and states that there is no means provided in either the art or the specification to administer these proteins to cells *in vivo*; nor means for controlling the activity of these proteins.

Applicants respectfully submit that suitable delivery methods to developed organisms are well known in the art, as set out above. Methods are known in the art for the delivery of proteins (for example, localized administration to tissues, e.g., via injection and targeting via cell-surface proteins). Methods of delivering DNA and RNA constructs (e.g., containing tissue-specific regulatory sequences) are also well known in the art. Accordingly, the skilled person would be well aware of methods for targeting cells in a developed organism.

Moreover, selective inhibition of cell proliferation/selective cell killing is enhanced in the present invention by use of the toxin/antitoxin system. According to the methods of the present invention, the provision of the toxin to target cells is accompanied by provision of the antitoxin to non-target cells. The ratio between the toxin and the antitoxin determines the effect on the cell. For example 1, in the paper by Slanchev *et al* (2005), provision of the toxin to target cells is accompanied by systemic provision of the antitoxin. The antitoxin is able to protect the non-target cells against low level, "leaky" expression of the toxin. Thus, the method of the present invention serves to refine the targeting, so as to provide selectivity for the target cells.

In view of the above, reconsideration and withdrawal of the rejection is requested.

Claims 1-4, 10 and 12-16 stand rejected under 35 USC 112, second paragraph, as allegedly being indefinite. Withdrawal of the rejection is submitted to be in order in view of the above-noted claim amendments and comments that follows.

The Examiner contends that claim 1 is rendered indefinite by the phrase "under appropriate control for selective cell cycle inhibition and/or killing of said target cells". The Examiner states that, as evidenced by Pimental *et al* (2005), "kid exerts a reversible cytostatic effect" and so it is not clear how using the kid toxin would lead to killing of target cells rather than cell cycle inhibition of cells.

Applicants respectfully point out that the paper by Pimentel *et al* (2005) reports that kid is cytostatic in bacteria. Applicants have shown that, in eukaryotes, kis and kid can be used to regulate cell death. Indeed, this is expressly stated in Pimentel *et al* (2005) (paragraph spanning first and second columns of page 3460):

> "Kis and Kid can also function in eukaryotes, and have been used to conditionally regulate cell proliferation and cell death in these organisms (de la Cueva-Méndez *et al* 2003)."

Thus, it is not believed that there is any lack of clarity in the claims.

The Examiner has also contends that the limitations engendered by the term "appropriate" are not clear, and questions what is meant for something to be "appropriate". Claim 1 has been amended to delete the term "appropriate" and to refer instead to "under control so as to obtain selective cell cycle inhibition". It is believed that the revision addresses the Examiner's concerns.

Finally, the Examiner contends that claim 4 is rendered vague and indefinite by the phrase "carried out on the human or animal body". It is believed that this aspect of the rejection is rendered moot by the above-noted amendments to claim 4.

Reconsideration is requested.

This application is submitted to be in condition for allowance and a Notice to that effect is requested.

Respectfully submitted,

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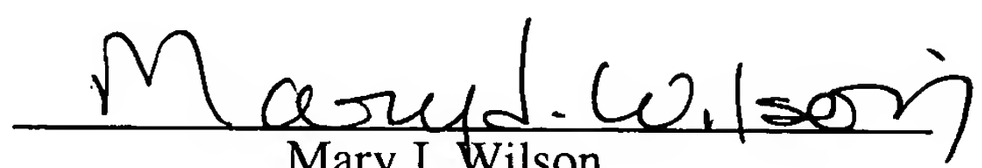
By: 
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